

CLAIMS

1. A method of liquefying starch-containing material, wherein the method comprises the steps of
 - 5 (a) treating the starch-containing material with a bacterial alpha-amylase at a temperature around 70-90°C for 15-90 minutes,
 - (b) treating the material obtained in step (a) with an alpha-amylase at a temperature between 60-80°C for 30-90 minutes.
- 10 2. The method of claim 1, wherein the starch-containing material is jet-cooking at 90-120°C, preferably around 105°C, for 1-15 minutes, preferably for 3-10 minute, especially around 5 minutes, before step (a).
3. The method of claims 1 or 2, wherein the starch-containing material is selected from the
 - 15 group consisting of: tubers, roots and whole grain; and any combinations of these.
4. The method of any of claims 1-5, wherein the starch-containing material is obtained from cereals.
- 20 5. The method of any of the claims 1-5, wherein the starch-containing material is selected from the group consisting of corn, cob, wheat, barley, rye, milo and potatoes; or any combination of these.
6. The method of claim 5, wherein the starch-containing material is whole grain selected
 - 25 from the group consisting of corn, wheat or barley or any combinations of these.
7. The method of any of the claims 1-8, wherein the starch-containing material is whole grain and said method comprises a step of milling the whole grain before step (a).
- 30 8. The method of any of the claims 1-5, wherein the starch-containing material is obtainable by a process comprising milling of whole grain.
9. The method of any of the claims 1-8, further comprising prior to step (a) the steps of;
 - i) milling of starch-containing material;
 - 35 ii) forming a slurry comprising the milled material and water.

10. The method of any of the claim 9, wherein the milling step is a dry milling step.

11. The method of any of the claims 9-10, wherein the milling step is a wet milling step.

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12. The method of any of the claims 1-11, wherein the starch-containing material is a side stream from starch processing.

13. The method of claims 1-12, wherein the bacterial alpha-amylase in step (a) is a *Bacillus* alpha-amylase, preferably derived from *Bacillus stearothermophilus* alpha-amylase or a variant with the mutations: I181*+G182* especially I181*+G182*+N193F.

14. The method of claims 1-13 wherein the alpha-amylase is step (b) is an acid alpha-amylase, preferably an acid fungal alpha-amylase, preferably derived from *Aspergillus* spp. preferably *Aspergillus niger* or *Aspergillus oryzae*.

15. The method of any of claims 1-14, wherein the acid alpha-amylase is an alpha-amylase having an amino acid sequence which has at least 70% identity to SEQ ID NO:1 preferably at least 75%, 80%, 85% or at least 90%, e.g., at least 95%, 97%, 98%, or at least 99% identity to SEQ ID NO:1.

16. The method of claims 1-15, wherein the acid alpha-amylase is an alpha-amylase having the amino acid sequence set forth in SEQ ID NO:1.

17. The method of claims 1-16, wherein the mash obtained after step (b) has a DE value of above 16, preferably above 18, especially above 20, such as a DE value in the range from 16 to 30, preferably in the range from 18 to 25.

18. A process of producing a fermentation product from starch-containing material by fermentation, said process comprises:

- (i) liquefying said starch-containing material as defined in any of claims 1 to 17;
- (ii) saccharifying the liquefied mash obtained;
- (iii) fermenting.

19. The process of claim 18, further comprising recovery of the fermentation product.

20. The process of claims 18 or 19, wherein the fermentation product is ethanol.

21. The process of any of claims 18 to 20, wherein the saccharification and fermentation is carried out as a simultaneous saccharification and fermentation process (SSF process).

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22. The process of any of claims 18 to 21, further comprising a step of;

(iv) distillation to obtain ethanol;

wherein the fermentation in step (iii) and the distillation in step (iv) is carried out simultaneously or separately/sequentially; optionally followed by one or more process steps for further refinement of ethanol.

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23. The process of claim 18, wherein a fermenting organism capable of fermenting sugars to ethanol is used in step iii).

15 24. The process of any of claims 18 to 23, wherein the fermenting organism in step iii) is yeast, such as a strain of *Saccharomyces spp.*, preferably a strain of *Saccharomyces cerevisiae*.

20 25. The process of any of the claims 18 to 24, wherein the fermentation is carried out in the presence of a carbohydrate-source generating enzyme.

26. The process of claim 25, wherein the carbohydrate-source generating enzyme is glucoamylase, preferably derived from a strain of *Aspergillus*, preferably *Aspergillus niger* or *Aspergillus awamori*, a strain of *Talaromyces*, especially *Talaromyces emersonii*, or a strain of *Athelia*, especially *Athelia rolfsii*

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27. The process according to any of claims 18 to 26, said process comprising the steps of;

1) liquefying starch-containing material in accordance with the liquefaction method of claims 1-17;

30 2) liquefying the material obtained in step 1) in the presence of an alpha-amylase having an amino acid sequence which has at least 70% identity to SEQ ID NO:1; and

3) saccharifying the material obtained in step 2) ;

4) fermenting;

wherein the steps 1), 2), 3) and 4) is performed in the order 1), 2), 3), 4) or wherein 4) is performed simultaneously to or following 3).

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28. The process of claim 27, wherein step 4) is carried out using a fermenting organism capable of fermenting sugars to ethanol.

29. The process of claim 28, wherein the fermenting organism is yeast, preferably a strain of *Saccharomyces spp.*, preferably a strain of *Saccharomyces cerevisiae*.

30. The method of any of claims 27 to 29, wherein the mash obtained in step 2) has a DE value of above 16, preferably above 18, especially above 20, such as a DE value in the range from 16 to 30, preferably in the range from 18 to 25.

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31. A process of producing syrup from starch-containing material, comprising
(a) liquefying starch-containing material in accordance with the liquefaction method of any of claims 1 to 17,
(b) saccharifying the liquefied material.

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32. The process of claim 31, wherein the syrup is selected from the group comprising glucose, maltose, fructose syrups, malto-oligosaccharides, and isomalto-oligosaccharides.

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